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CHEMOTHERAPY IN MALARIA*

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Most of the recent advances in the chemotherapy of the malarias can be presented in the form of a commentary on the program of studies carried out under the auspices of the Office of Scientific Research and Development during the past four years. The studies were initiated in 1941 through the agency of the Committee on Medical Research with the aid of committees set up in the Division of Medical Sciences of the National Research Council.[1,2] The committees concerned with malaria underwent changes with the progressive expansion of the scope of the studies until, in the winter of 1943-44, the present program was evolved under the sponsorship of the Board for the Coordination of Malarial Studies and its various Panels. The Board is a coöperative endeavor and involves the participation of the Committee on Medical Research, National Research Council, Army, Navy, United States Public Health Service, and a group of investigators in civilian institutions under con-

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The information contained in this paper is largely derived from work now sponsored by the Board for the Coordination of Malarial Studies and carried out by investigators of various institutions through contracts between them and the Office of Scientific Research and Development which were recommended by the Committee on Medical Research.

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tract with the Office of Scientific Research and Development.

The primary purpose of the investigations has been, at all times, to satisfy specific needs of the armed services. Information was required which would permit the better management of the malarial hazard first, as a factor of operational significance to combat troops in hyperendemic areas, and second, as a problem relating to the maintenance of good health of troops while they were in and after they had been removed from such an area. It was apparent from a consideration of these needs that initially, considerable work must be performed with antimalarial agents already available so that they could be used to the best advantage. However, it also seemed likely, that advantages would accrue were new and more effective antimalarials developed. More specifically, it was hoped that among the newer agents there would be one or more which would either prevent malarial infections or effect definitive cures at well tolerated dosage.

The direction of the early clinical work (1942-1943) was conditioned largely by the early loss to the United Nations of their normal sources of supply of quinine and by the lack of an adequate stockpile. Those who were intimately concerned with the malarial problem during the first year of the war will recall the gravity of the situation. The worry which was incidental to the lack of an adequate supply of quinine was enhanced by the preliminary reports from the field which carried the suggestion that quinacrine was of little use in the suppression and treatment of the malarias and that pamaquin as then used had little value in the cure of vivax malaria.

The Investigation of Quinacrine: Quinacrine was said to be unable to produce a prompt termination of the clinical attack, more particularly, in falciparum malaria, much less a cure in either falciparum or vivax malaria. In addition, it was reported to be both highly toxic and relatively ineffective when used as a suppressive. These experiences caused many medical and line officers to believe that the control of malaria by quinacrine was not practicable. The clinical results were at such variance with reports in the literature[3] that an uncertainty existed in the minds of some as to the chemical identity of German and American quinacrine. This uncertainty was sufficiently serious to require the services of a number of chemists, pharmacologists and clinicians, the integrated efforts of which established the identity of the two products by the summer of 1942.[4] Even so there was a growing belief

that quinine was the only known antimalarial agent of consequence, that such of it as was available must be used sparingly,[5] and that a substitute for quinine, with all its limitations, was not only highly desirable, but urgently needed.

The initial confusion concerning the therapeutic efficacy of quinacrine resulted from the lack of information which would permit its use in a rational manner. Empirical dosage schedules had been established at an earlier date and were generally used by the services.[6] These were based upon studies performed in the 1930's on groups of individuals with varying degrees of acquired immunity.[3] The dosage schedules which were found to be effective in such individuals are now known to be generally ineffective when applied to those who are wholly susceptible to these diseases. Furthermore, the recommended dosage regimen of suppressive quinacrine therapy brought into sharp focus the gastrointestinal irritation which frequently accompanies the oral administration of doses larger than 0.1 gram for suppressive purposes.

It is now established that quinacrine is a generally useful and highly effective antimalarial, quite superior to quinine in most situations.[7] This important advance in our knowledge resulted from the development of chemical methods for the estimation of quinacrine in biological fluids and tissues[8, 9] and the use of these methods in studies which defined the antimalarial activity and physiological disposition of the drug.

It was first demonstrated in the winter and spring of 1943 that the inherent antimalarial activity of quinacrine is high and is related to its concurrent plasma concentration,[10] and that quinacrine is very extensively localized in the tissues of the body and degraded and excreted at low rates. It seemed reasonable to believe that the efficacy of suppressive quinacrine therapy would depend more upon the total dosage administered per week than upon the specific pattern of the dosage regimen. It also seemed reasonable to believe that, since a quick therapeutic effect only obtains when a high plasma quinacrine concentration is reached early in the course of therapy, the efficacy of quinacrine in causing an abrupt termination of a clinical attack would depend largely upon the amount of drug administered during the first 24 hours of therapy.[11, 12]

These concepts were quickly translated into practical regimens of therapy which have been in use by the Army and Navy for some two years.[13] As the result of this experience, it is known that effective sup-

pression of malaria in the adult requires the administration of 0.1 gram of quinacrine daily; also to derive the maximal benefit, the drug should be administered for some two weeks prior to entry into an hyperendemic area and for some weeks after the risk of infection no longer exists. Prompt control of the clinical attack in vivax and falciparum malaria and cure in the latter infection can be accomplished by the administrtaion of 0.8 to 1.0 gram of quinacrine in divided doses during the first 12 to 18 hours of treatment and 0.1 gram three times daily for an additional six days, or, an equivalent amount in a shorter period of time.

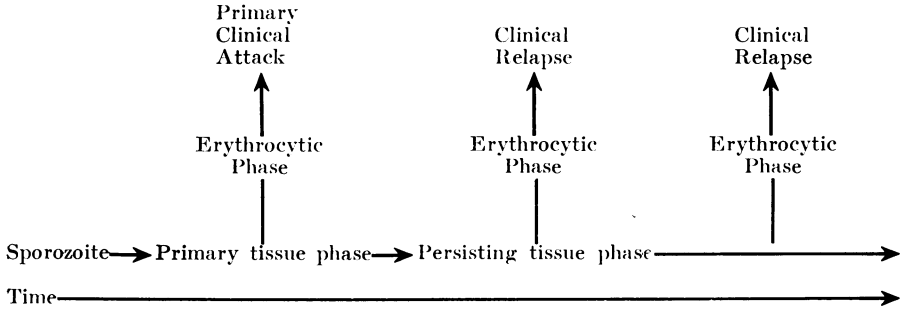
The establishment of quinacrine as a highly effective routine antimalarial permitted the clinical facilities of the civilian program to be directed towards the attainment of other objectives. This was because the proper usage of quinacrine had largely removed malaria from the field of tactical importance to the Services.

A consideration of the subsequent studies, which were aimed very specifically at the development of a curative agent in vivax malaria, requires a definite understanding of the specific limitations of quinacrine. It will prevent the inception of clinical falciparum malaria when given as a suppressive, and effect a prompt and definitive cure when the infection is once established. However, quinacrine will not prevent the inception of vivax malaria although it is highly effective in suppressing its clinical manifestations, and, it will not effect a definitive cure when the infection is once established. These two limitations are fundamental in nature. The drug has other limitations but these are of lesser importance. It is prone to cause gastrointestinal reactions when administered for suppressive purposes in single doses in excess of 0.1 gram. This is of some consequence since it increases the administrative burden of suppressive therapy, requiring as it does a daily dose of drug. The yellow staining of the skin which is a constant finding with quinacrine therapy may also be mentioned since it is an undesirable feature. Other toxic manifestations are too infrequent to warrant special attention at this time.

The Search for new more Effective Antimalarials (Suppressives): An understanding of the biological basis for the most important fundamental limitation of quinacrine, i.e., its inability to cure vivax malaria, and the design of experiments to discover antimalarials not having a similar limitation require an appreciation of some of the important features of the biology of the malaria infections. The disease mechanisms

FIGURE I

PROPOSED SCHEME TO DESCRIBE THE UNDERLYING DISEASE MECHANISM OF VIVAX MALARIA



which underly the human infections may be assumed to parallel, in certain respects, those which have been described in some detail for several avian infections.[14] A general outline of a mechanism which seems reasonable for vivax malaria[12] is presented in the accompanying figure (Figure 1). Consideration is not given to the sexual forms of the parasite, i.e., the gametocytes. These have no importance in conditioning the clinical course of the disease in the individual patient.

Such an outline assumes that, subsequent to the deposition of sporozoites by the mosquito, a tissue phase of the developing plasmodium is established and persists for a considerable period of time. It is further assumed that lines of erythrocytic forms of the parasite are derived from the tissue phase as discrete episodes in the course of the disease. These in turn are responsible for the initial attack and the parasitological and clinical relapses which are characteristic of vivax malaria. The early portion of the disease mechanism in falciparum malaria appears to be similar to that of vivax malaria in all respects. However, there is no need to assume the life history of the falciparum parasite also includes a persisting tissue form since this type of malaria does not systematically relapse. If a persisting tissue form is present in falciparum malaria, then it poses no special therapeutic problem since, contrary to vivax malaria, the disease can be cured by a variety of chemotherapeutic agents which are also highly effective against the erythrocytic forms of the plasmodium.

It will be appreciated from such a summary, that the malarial parasite has a rather complex life history within the human host when the infection is acquired naturally. This involves several phases of develop-

ment each of which may be expected to differ from the others as to morphology, metabolic characteristics, and the cell type of the host within which it takes place. It will also be appreciated that potentially there are several types of antimalarial activity in the vivax infection. For convenience these may be designated as prophylactic if the action is on the sporozoite or the primary tissue forms of the plasmodium, as suppressive if on the erythrocytic forms or as curative if on the persisting tissue forms.

Each of these types of antimalarial activity may be examined separately. The testing for prophylactic or curative activity requires the use of mosquito induced malaria. The latter is further complicated by the need for a long term observation period before a final judgment can be made on the extent to which a given drug or a given dosage regimen is curative. However, suppressive antimalarial activity is amenable to simple and rather precise assay by the use of blood induced infections.[15] This type of infection has a more simple disease mechanism. It is established by the simple transfer of infected blood from a patient with an active infection and having only the erythrocytic phase of the disease; it permits the examination of the suppressive type of activity alone. It is important to note that the differences in suppressive and curative activities could be either quantitative or qualitative. Consequently, the development of an antimalarials with a high degree of curative action in vivax malaria could be approached with reason by more than one way.

One approach could be based upon an hypothesis which held the fundamental metabolic organization of the persisting tissue forms of *P. vivax* to be essentially the same as that of the erythrocytic forms of the plasmodium. Due to differences in their environments, the tissue form was assumed to be less susceptible to the antimalarial effect of drugs such as quinacrine. Accordingly, a reasonable approach to the development of curative agents appeared to lie in the direction of obtaining more active drugs as evidenced by their ability to exert an action upon the erythrocytic forms of the parasite, i.e., as manifested in the blood-induced infection. It was hoped that if the intensity of this type of antimalarial activity was sufficiently great in the case of any drug then it would not only interrupt the erythrocytic phase of the vivax parasites but would also obliterate the persisting tissue phase of a naturally acquired infection and so cure the disease.

Another approach could be based upon an hypothesis which held

the fundamental metabolic organization of the persisting tissue forms of *P. vivax* to be different from that of the erythrocytic forms of the plasmodium, at least insofar as the susceptibilities of their essential biological systems were concerned. Accordingly a chemotherapeutic agent might affect the tissue forms through an action which was qualitatively different from any which produced a dramatic effect upon the asexual forms in the erythrocyte, i.e., the blood-induced infection had little value in the search for curative agents. In accordance with this hypothesis, it would be quite possible to miss a curative agent unless a number of representatives of each group of chemicals studied were examined for curative action. Compounds could then be selected for this type of activity because of special activities other than suppressive in the avian infections, or, they could be screened for curative action in the human vivax infection without prior experimental trial.

It was generally agreed in the fall and winter of 1943 that, sufficient evidence was not available for one to decide which of these two hypotheses was more reasonable. Consequently, there was considerable discussion, as to whether blood-induced infections could be of value in a program, the end of which was the development of curative agents for vivax malaria. However, this type of infection was continued in use on a rather extensive scale, its use being based on the tentative acceptance of the reasonableness of the first working hypothesis. As a logical consequence and as the major effort at that time, a systematic attempt was made to increase antimalarial activity in a number of the chemical series then under exploration and the best representative in each series was selected on the basis of information from blood-induced infections, and examined for curative action in mosquito induced vivax malaria. Actually the overall procedure adopted represented a partial compromise between the two working hypotheses. Certain of the compounds studied for curative action had relatively little suppressive action, their selection being based upon two considerations. They were representatives of chemical series as yet untried for curative action and although they might have had little suppressive activity, the compound tested was better in this respect than the other members of the series examined: In addition any compound showing a special type of activity in the avian infections, such as curative or prophylactic, was also tried for curative action in vivax malaria.

The advantages of this approach, at that stage of the program, were

three. First, it was believed likely, with the leads then available, that suppressive antimalarial activity could be increased many fold in several different types of compounds and, as the result of this effort, compounds would shortly become available with which to test the correctness of the first working hypothesis. Second, it would permit the study of a number of chemical series, as yet unexamined, for their possession of curative action and perhaps establish a correlation between special activities in avian infections and curative activity in vivax malaria. Third, it seemed reasonable to suppose that this approach to the problem would result in the development of antimalarials superior to quinacrine although they might have the same fundamental limitations. The third possibility was important. It was desirable to have available antimalarials other than quinacrine should the long-term continuous administration of quinacrine to the human be accompanied by toxic manifestations which at the time could not be predicted.

It was early demonstrated beyond doubt that the suppressive antimalarial activity of a compound, when measured in a single avian infection, may have little prediction value for the situation obtaining in the suppression of peripheral parasitemia in the human malarias.* It was later demonstrated that the sum total of the information, when derived from the study of the activity of a compound or series of compounds in several avian infections using several avian hosts, does have fair prediction value in the selection of compounds for trial as suppressives in the human malarias. Lastly it was demonstrated, within the compounds studied, that none had higher antimalarial activity of a suppressive nature in both human infections that had been observed in at least one of the avian infections.** This information was accumulated incidental to the survey of a very large number of compounds (ca. 14,000) for activity in the avian infections, the survey of a limited number of compounds (ca. 65) for suppressive activity in the human infections and a selected number of the latter group (ca. 20) for prophylactic and curative action in vivax malaria.

Out of these extensive studies no compounds were developed with prospects of being useful as curative agents in vivax malaria although

* The data available on antimalarials at the beginning of the program and those from allied fields of chemotherapy led Doctor E. K. Marshall, Jr., Chairman of the Pharmacological Panel, to this conclusion.

** The substantiation of these general concepts was as the result of the combined efforts of all Office of Scientific Research and Development contractors on both the pharmacological and clinical levels. The experimental facts themselves will be contained in a monograph entitled "A survey of antimalarials 1941-1946," edited by Doctor F. Wiselogle and prepared by The Office for the Survey of Antimalarial Drugs. This monograph should be available by the summer or fall of 1946.

several have unquestioned advantages over quinacrine and quinine. For example, a plasma quinine concentration of 5 mg. per liter maintained for four days terminates a blood induced infection or a clinical attack of a mosquito induced infection due to the McCoy strain of *P. vivax*.[10] An equivalent effect will be produced by 30 micrograms per liter of quinacrine.[10] However, plasma concentrations of quinine in excess of 12 mg. per liter and of quinacrine in excess of 150 micrograms per liter are not generally well tolerated.

Of the newer compounds developed during the fall and winter of 1944-1945, there is one (SN-7618)* which is rather well tolerated at dosage schedules which produce plasma drug concentrations some 30 times those required to terminate the clinical attack.[16] Others, where activities have been placed less precisely in terms of plasma drug concentrations, can be administered in daily doses many many times those required to produce a demonstrable antimalarial effect. Nevertheless, these agents have nothing to offer as curative agents in vivax malaria. It may be concluded from these observations that the major working hypothesis selected for trial in 1943 in the attempt to develop curative agents in vivax malaria is not correct.

Before considering the next phase of studies it will be of some interest to take note of the potentialities of certain of the agents which, at least to some extent, are by-products of an unsuccessful attempt to produce a curative agent for vivax malaria. Among the more promising compounds are some which may be expected to effect complete suppression when administered once weekly in a well tolerated dose. They will also effect an abrupt termination of a clinical attack of vivax and a cure of falciparum malaria when administration is limited to one or at most, two days. None of these highly effective agents has, as yet, been fully exploited. However, information is at hand which permits the prediction that they will constitute a relatively simple means for the complete control of malaria in many areas due to the lessening of the administration problem of suppressive therapy as compared to quinacrine. They may also, in specific areas, contribute to the eradication of the malarias through their ability to curtail transmission of the disease. Exploration of the advantages to be derived from the use of some of these newer agents is now under way.

* SN-7618 is *7-chloro-4-(4-diethylamino-1-methylbutylamino)* quinoline. Several closely related compounds have comparable activity.

The Search for More Effective Antimalarials (Curative): Of importance to the attainment of one of the ultimate objectives of the program, was the conclusion that a simple increase in antimalarial activity as evidenced by an effect against the erythrocytic forms of the parasite cannot, *per se*, be expected to lead to curative drugs for vivax malaria. The obtaining of this information marked the beginning of the present stage of the malaria studies. This has been characterized by the direct approach to the problem of devising curative agents which are now assumed to require qualitatively different actions than those which are simply reflected in a reduction of peripheral parasitemia in vivax and falciparum malaria. These studies are proceeding in several laboratories and with some prospect of success.

A serious obstacle to success in this endeavor stems from the fact that, with our present knowledge, it is not possible to use the experimental avian malarias effectively to screen compounds prior to their selection for trial as curative agents in vivax malaria. Drugs have been developed which possess prophylactic and/or curative action in one or another of the avian infections but, generally speaking, these actions are not a reflection of a similar action in vivax malaria. Actually, there is, as yet, no general correlation between these special actions in the avian infections and comparable action in the human infections. The promise that curative drugs will eventually be found stems solely from the recent confirmation of the earlier studies on the curative action of pamaquin, an 8-aminoquinoline.

It seems reasonably certain that the older investigations on the antimalarial activity of pamaquin led to conclusions which are essentially correct.[17] That is, pamaquin, when administered at high dosage, has a curative action in vivax malaria when administered concurrently with quinine over a long period of time. This is a fact of importance. It demonstrates that the persisting tissue forms of the plasmodia which are held to be responsible for the relapse in vivax malaria are subject to the lethal action of a drug to which the type of host cell within which they reside is not also generally susceptible. Furthermore, the curative action of pamaquin makes available a specific lead towards the synthesis of better tolerated curative agents.

This synthetic lead is now being explored extensively. It did not receive attention earlier in the program for three reasons. It was known that pamaquin analogs had received systematic study by the Germans,

French, and Russians, both before and after the development of pamaquin, and no better drug had been announced. It was also known that pamaquin and many of its analogs possess seriously toxic effects when administered at a dosage well below that which is generally curative. Finally, it was hoped that a curative agent might be found in other series of substances which were characterized by lesser toxicity. It was not until the last possibility seemed unlikely, at least in the near future, that it was deemed advisable to embark upon an extensive study of 8-aminoquinolines.

It is now known that previous exploration of the 8-aminoquinolines was inadequate to be certain that pamaquin is the best drug to be derived from this series. Furthermore, the careful study of the antimalarial activity and toxicity of pamaquin and a selected series of 8-aminoquinolines seems to indicate that such an exploration will be profitable. A consideration of what has been done with other chemical series, whereby one or another aspect of antimalarial activity has been greatly increased without a comparable increase in toxicity, makes the prospect of obtaining a useful agent from this group of substances rather bright though highly speculative.

Should it happen that generally useful curative agents for vivax malaria are contained in the series of 8-aminoquinolines, this will make an interesting chapter in the development of antimalarial agents. Pamaquin was first used on a very extensive scale in the late 1920's. It was said, on the basis of preliminary studies, to possess some suppressive effect upon the erythrocytic phase of vivax malaria, as well as a curative action in this disease. It was also said to have little or no suppressive effect upon the erythrocytic phase of falciparum malaria but a dramatic effect upon the gametocytes of the plasmodium responsible for this infection. In addition it was said to be a true prophylactic in each infection.

Shortly after the drug was available for large scale experimental use it became apparent that the dosage recommended in the earlier studies could be expected to produce widespread and seriously toxic effects. However, the extent of the toxicity was not appreciated at a sufficiently early date to prevent the organization of reasonably well controlled trials to assay the curative action of pamaquin in vivax malaria.

It was common practice at that time to treat both vivax and falciparum malaria with full therapeutic doses of quinine, about 2.0 grams

daily, for some 14 to 21 days. Consequently, when pamaquin was tested it was also administered for this period of time. It was demonstrated quite early that a combination of concurrently administered pamaquin and quinine is superior to the administration of either drug alone. For example, in one series[17] there was observed 75 per cent relapses in an eight weeks period with quinine alone, 25 per cent with pamaquin alone and essentially none with a combination of the two antimalarials. The dosage of pamaquin in these studies, calculated as the hydrochloride was as low as 40 mg. daily in many patients. It is possible that such a dosage regimen would have come into general use despite the toxic effects of the pamaquin except for the discovery and limited exploration of quinacrine which occurred about this time.

It was found, with quinacrine, that little advantage is derived from the administration of quinacrine beyond a seven day period of treatment at 0.1 gram three times a day in population groups having a fair degree of immunity. As a reaction to this finding, the belief gained credence that quinine administration might also be curtailed to a similar period with advantage. This belief together with a growing appreciation of the toxic hazard of pamaquin led to a curtailment in the duration of the administration of quinine as well as a lowering of the pamaquin dosage commonly used. It is not surprising then, that the publications of the League of Nations recommended that combination pamaquin and quinine therapy be limited to seven days with the daily dose of pamaquin no higher than 30 mg. of the hydrochloride. The increase in the toxicity of pamaquin when administered concurrently with quinacrine led to the adoption of a convention whereby pamaquin was administered in similar dosage but for only five days and separated from quinacrine administration by a three day drug free interval. It is now known that pamaquin administered at such a dosage and in such manner has little to offer as a curative agent in vivax malaria although it is a highly effective gametocidal agent. It seems likely that the early studies with pamaquin produced a different therapeutic result because the dosage was usually higher, administration was for a longer period of time and the drug was administered concurrently with quinine. However, all three features were lost sight of in the years between 1931 and 1941 so that, the early recommendations for the use of pamaquin by the services were not of the type that would be expected to yield a significant proportion of cures.[6]

Work during the past year has retraced these steps and it is believed that the examination of the curative action of the 8-aminoquinolines is again on a sound experimental basis. However, progress towards the ultimate goal of these studies will be slow since, at the moment, it is necessary to restrict the primary examination of compounds in this series for curative action to human experimental material.

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